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Production of acetone, butanol and ethanol by *Clostridium beijerinckii* BA101 and *in situ* recovery by gas stripping

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Summary

We examined the effect of gas-stripping on the *in situ* removal of acetone, butanol, and ethanol (ABE) from batch reactor fermentation broth. The mutant strain (*Clostridium beijerinckii* BA101) was not affected adversely by gas stripping. The presence of cells in the fermentation broth affected the selectivities of ABE. A considerable improvement in the productivity and yield was recorded in this work in comparison with the non-integrated process. In an integrated process of ABE fermentation-recovery using *C. beijerinckii* BA101, ABE productivities and yield were improved up to 200 and 118%, respectively, as compared to control batch fermentation data. In a batch reactor *C. beijerinckii* BA101 utilized 45.4 g glucose l^{-1} and produced 17.7 g total ABE l^{-1}, while in the integrated process it utilized 161.7 g glucose l^{-1} and produced total ABE of 75.9 g l^{-1}. In the integrated process, acids were completely converted to solvents when compared to the non-integrated process (batch fermentation) which contained residual acids at the end of fermentation. *In situ* removal of ABE by gas stripping has been reported to be one of the most important techniques of solvent removal. During these studies we were able to maintain the ABE concentration in the fermentation broth below toxic levels.

Introduction

Butanol, an important industrial chemical, is currently used as a feedstock in the plastic industry. It is also used as a food-grade extractant in the food and flavour industry. Acetone and butanol were used as solvents and in the production of the rubber monomers, butadiene, and dimethyl butadiene (Mollah & Stuckey 1993). Butanol can also be used as a fuel extender, which has several advantages over other fermentatively produced fuels and these include high energy content, miscibility with gasoline, and diesel fuel, and a low vapour pressure (Qureshi & Blaschek 1999a). The renewal of interest in the microbial production of butanol [often called acetone, butanol, ethanol (ABE fermentation)] is based almost exclusively on the awareness of fossil fuel depletion and the uncertainty of petroleum supplies. *Clostridium beijerinckii* BA101 is able to ferment a variety of carbohydrates to butanol, including apple waste and starch packing peanuts (Jesse *et al.* 2002). Butanol has great potential because it has many characteristics which make it a better fuel extender than ethanol. Additionally, fermentation-derived butanol is preferred in the food industry (for food flavour extraction) as petroleum-derived butanol has the potential for carcinogen carryover (Formanek *et al.* 1997). For these two important reasons, bio-based butanol is preferred. Production of butanol by fermentation can also help the US with respect to independence from foreign oil.

The main problem associated with the ABE fermentation by *C. acetobutylicum* and *C. beijerinckii* is butanol toxicity/inhibition to the culture. To solve this problem, various alternative *in situ*/online butanol removal techniques including membrane-based systems, such as pervaporation (Groot *et al.* 1984; Qureshi *et al.* 1992, 1999, 2001), perstraction (Qureshi *et al.* 1992), reverse osmosis (Garcia *et al.* 1986), adsorption (Ennis *et al.* 1987; Nielson *et al.* 1988), liquid–liquid extraction (Evans & Wang 1988) and gas stripping (Groot *et al.* 1989) have been examined. The application of some of these recovery techniques can allow the use of concentrated sugar solutions in the fermentor (Maddox *et al.* 1995; Qureshi & Blaschek 2001) thereby reducing volumes of the process streams. In such systems, up to 100% utilization of the sugar available in feed has been demonstrated. It should be noted that membrane-based

systems demonstrate high selectivities for ABE but may suffer from fouling and clogging. On the other hand, gas stripping is a technique which allows for the selective removal of volatiles from fermentation broth and uses no membrane. The volatile properties of the ABE permit easy product removal by gas stripping. Gas can be sparged into the fermentor through a rotating fermentor shaft and volatiles can be condensed and recovered from the condenser. The gas-stripping process has a number of advantages over other removal processes, for example: it is simple and inexpensive to operate and does not suffer from fouling or clogging due to the presence of biomass. Ennis *et al*. (1986) were the first to investigate the application of gas stripping in butanol fermentation using *C. acetobutylicum*. Gas stripping has been described as one of the most important techniques for removing butanol from the fermentation broth (Maddox 1988; Groot *et al*. 1992).

C. beijerinckii BA101 is a genetically manipulated strain which has the potential for becoming an industrial microorganism for producing butanol from corn and other substrates. It has been reported that this strain is able to produce up to 32.6 g l^{-1} total ABE (Chen & Blaschek 1999) under optimized conditions as opposed to 20 g l^{-1} by other solventogenic cultures (Maddox 1988). While *C. beijerinckii* BA101 stops growing above 165 g l^{-1} (Qureshi & Blaschek 1999a), *C. acetobutylicum* P262 can tolerate up to 227 g sugar l^{-1} (Qureshi & Maddox 2003). This observation is an important difference between *C. acetobutylicum* P262 and *C. beijerinckii* BA101. Since *C. beijerinckii* BA101 is a mutant (and different) strain, butanol removal studies carried out using other solventogenic strains may not be applicable for *C. beijerinckii* BA101. For this reason, the present work is considered novel. The authors are aware of the gas-stripping work performed by various investigators. It is also known that butanol-producing cultures are negatively affected by mechanical agitation. *In situ* gas stripping causes vigorous agitation of culture fluid (depending on gas recycle rate), and hence may negatively affect *C. beijerinckii* BA101. Additional objectives were to study the effect of cell concentration on ABE selectivity and the use of concentrated sugar solutions for butanol production. In batch reactors, a sugar concentration more than 60 g l^{-1} cannot be utilized, due to product inhibition caused by butanol.

Materials and methods

Organism, culture maintenance and fermentation conditions

The *C. beijerinckii* BA101 culture was generated using *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) together with selective enrichment on the non-metabolizable glucose analog 2-deoxyglucose (Annous & Blaschek 1991). Laboratory stocks of *C. beijerinckii* BA101 were routinely maintained as spore suspensions in sterile double distilled water at 4 °C. *C. beijerinckii* BA 101 spores (200 μl) were heat shocked for 10 min at 80 °C followed by cooling for 5 min prior to inoculation. Heat shocked spores were inoculated into 20 ml Tryptone-glucose-yeast extract (TGY) medium (in 50 ml screw capped Pyrex bottle) which was incubated anaerobically in the anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) for 15–16 h at 36 ± 1 °C until active growth was observed.

Batch fermentation

A 2-l bioreactor (New Brunswick Scientific Co., New Brunswick, NJ) was used throughout these studies. In all experiments, the temperature was maintained at 35 °C in the absence of agitation or pH control. Fermentor containing 60 g glucose l^{-1} and 1 g yeast extract l^{-1} (1.6 l reaction volume) was sterilized at 121 °C for 15 min. On cooling to 35 °C under an oxygen-free nitrogen atmosphere, filter-sterilized P2 stock solutions [(buffer: KH_2PO_4, 50 g l^{-1}; K_2HPO_4, 50 g l^{-1}; Ammonium acetate, 220 g l^{-1}), (vitamin: Para-amino-benzoic acid, 0.1 g l^{-1}; thiamin, 0.1 g l^{-1}; Biotin, 0.001 g l^{-1}), (mineral: $MgSO_4 \cdot 7H_2O$, 20 g l^{-1}; $MnSO_4 \cdot H_2O$, 1 g l^{-1}; $FeSO_4 \cdot 7H_2O$, 1 g l^{-1}; NaCl, 1 g l^{-1})] (Qureshi & Blaschek 1999b) were added, followed by the inoculation of the bioreactor with 5% (vol/vol) highly motile cells of *C. beijerinckii* BA101. Oxygen-free nitrogen gas was swept over the headspace of the fermentor until the culture produced its own gases (CO_2 and H_2). Samples were withdrawn at various intervals for analysis.

Gas-stripping

The medium used in this technique was the same as that used for batch fermentation experiments except that different glucose concentrations (60–161.7 g l^{-1}) were used and the reaction volume was 1.0 l until unless otherwise stated. Batch fermentations were allowed to proceed for 15 h (glucose 60 g l^{-1}) and 36 h (glucose 161.7 g l^{-1}) when the ABE concentrations approached 3–4 g l^{-1}, after which gas-stripping was applied. During gas-stripping stringent anaerobic conditions were maintained using oxygen-free nitrogen gas. The gases (CO_2 and H_2) were recycled (3000 ml min^{-1}, unless otherwise stated) through the system using a twin-head peristaltic pump. The ABE vapors were condensed using a cooling machine (GeneLine; Beckman Instruments Inc., Palo Alto, CA, USA) and a condenser (62 × 600 mm and cooling coil surface area 1292 cm^2) to −2 °C. In order to cool, ethylene glycol (50% v/v) was circulated at a flow rate of 600 ml min^{-1} through the condenser. The stripped ABE were pumped into the solvent collector using a peristaltic pump. Oxygen-free distilled water was added at intervals into the reactor to maintain a constant liquid level (to compensate for water loss due to gas-stripping) inside the reactor. A schematic diagram of the process is shown in Figure 1. Temperature in the

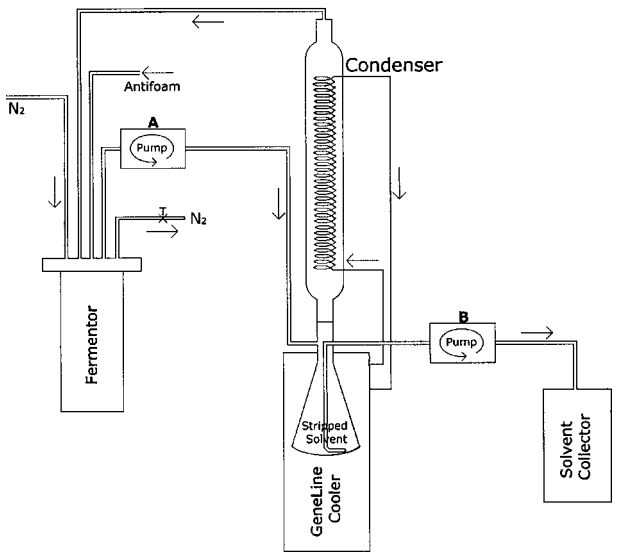

Figure 1. A schematic diagram of butanol production and *in situ* recovery by gas stripping. Pump A: gas recycle pump; pump B: condensed solvent removal pump.

reactor was controlled at 33–35 °C and there was no pH control during the entire process. Antifoam 204 (Sigma Chemicals, St. Louis, MO, USA) was added manually as required.

Analytical procedures

Cell concentration was estimated by optical density and cell dry weight using a predetermined correlation between optical density at 540 nm and cell dry weight. ABE and acids (acetic and butyric) were measured using a 6890 Hewlett–Packard gas chromatograph (Hewlett–Packard, Avondale, PA) equipped with a flame ionization detector (FID) and 2 m × 2 mm glass column (10% CW-20M, 0.01% H_3PO_4, support 80/100 Chromosorb WAW). Productivity was calculated as total ABE concentration (g l^{-1}) divided by fermentation time (h). Fermentation time was defined as the time when the fermentation ceased or glucose was depleted. Yield was defined as total grams of ABE produced per total grams of glucose utilized. Selectivity was calculated as $\alpha = [y/(1-y)]/[x/(1-x)]$, where x and y are weight fractions of ABE in fermentation broth and condensate, respectively. The rate of glucose utilization was defined as g of glucose utilized over a given time interval. The rate of solvent production was defined as ABE (g) produced for a given time interval.

Glucose concentration was determined using a hexokinase- and glucose-6-phosphate dehydrogenase (Sigma Chemicals, St. Louis, MO, USA)-coupled enzymatic assay. The fermentation broth was centrifuged (microfuge centrifuge) at 14,000 rev/min for 3 min at 4 °C. A portion of the supernatant (10 μl) was mixed with glucose (HK) 20 reagent (1.0 ml) and incubated at room temperature for 5 min. Standard solutions of anhydrous *d*-glucose containing 1, 2, 3, 4 and 5 g of glucose l^{-1} of distilled water were prepared. 10 μl of each of the standard solutions was mixed with glucose (HK) 20 reagent (1.0 ml) and incubated at room temperature for 5 min. A blank (deionized water) (10 μl) was incubated with the reagent and was used for zero adjustment of the spectrophotometer. After 5 min, the absorbance was measured at 340 nm using a Beckman DU 640 spectrophotometer and the glucose content in the sample was computed by least squares linear regression using a standard curve.

Results

Characterization of the gas-stripping process

In order to find rates of ABE removal under fermentation conditions at various ABE concentrations, a gas-stripping experiment was conducted at 35 °C using model ABE solution. Nitrogen gas was used to strip ABE at a gas flow rate of 4.6 l min^{-1}. The initial concentrations of ABE in the model solution were 6.8, 15.9 and 0.5 g l^{-1}, respectively. During stripping, ABE concentrations at various times are shown in Figure 2a. Removal of butanol followed a curve while removal of acetone and ethanol followed straight lines. It should be noted that under the present conditions the rate of

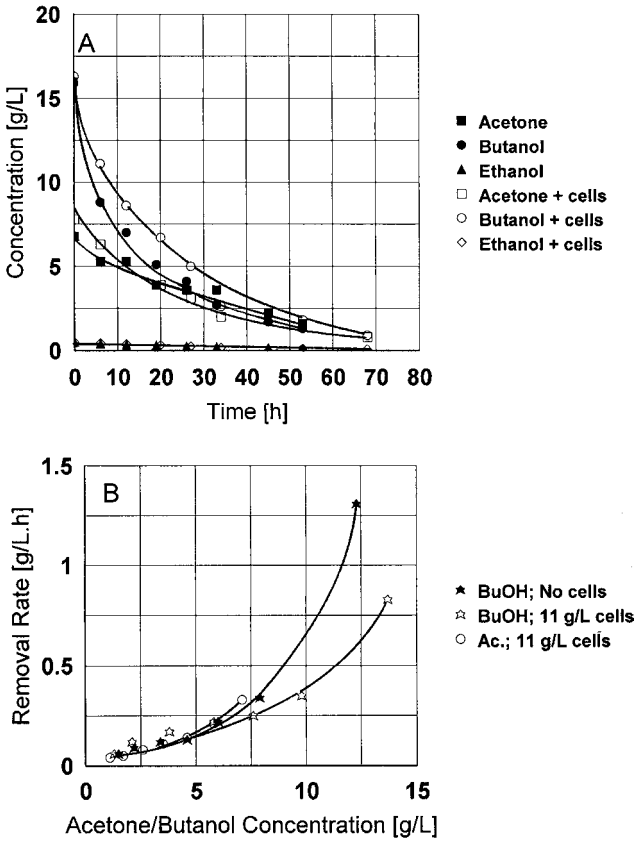

Figure 2. Removal of butanol and acetone from model solutions. (A) Butanol, and acetone concentration vs. time; (B) Rate of removal of butanol, and acetone at various butanol, acetone concentrations.

removal of butanol was concentration-dependent (Figure 2b), whereas for acetone and ethanol these rates were constant (not shown). The rates of removal of acetone and ethanol were 0.06 and 0.01 g l^{-1} h^{-1}, respectively, under the present conditions. During the gas-stripping experiment all the three solvents were selectively removed and their selectivities are shown in Figure 3. Within a concentration range of 1.5–12.3 g l^{-1} butanol selectivities were 13.83–10.26. Acetone and ethanol selectivities ranged from 4.12–6.42 (Figure 3b) and 4.90–7.90 (Figure 3c), respectively. It should be noted that while butanol and ethanol selectivities had no trend (scattered points), acetone selectitvities followed a straight line. The objective behind presenting the scattered data points was to inform the reader that in gas-stripping experiments scattered selectivities were obtained within a range. Even with the scattered points, a comparison can be made between selectivities using model solutions and fermentation broths.

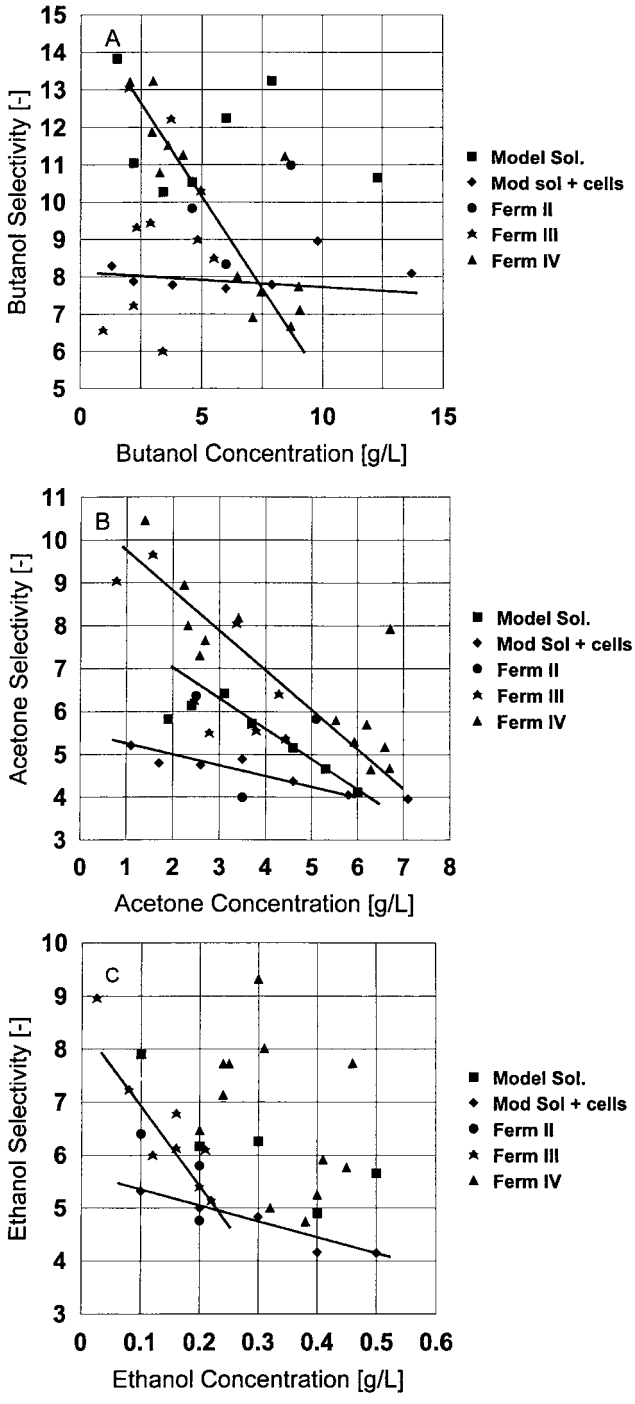

Figure 3. Butanol, acetone, and ethanol selectivities during their removal from model solution and fermentation broth. (A) Butanol selectivities; (B) Acetone selectivities and (C) Ethanol selectivities.

Fermentation broth is viscous due to the presence of cells. For that reason the rate of removal of ABE from fermentation broth may be different than that of the model solution used above. Hence an experiment was conducted with the same initial ABE concentrations and 11 g cells l^{-1}. In this case the rates of removal of butanol and acetone are shown in Figure 2b. The data suggest that presence of cells adversely affects the butanol removal rate above 7.5 g butanol l^{-1} concentration in the solution. As opposed to the model solution in the absence of cells, rate of removal of acetone varies with acetone concentration (Figure 2a, b). These data suggest that presence of cells affects the removal rate of acetone too. It should be noted that selectivities of butanol were found to be significantly reduced in the presence of cells (Figure 3). Selectivities of ethanol were also reduced in presence of cells. In presence of cells selectivities of all three solvents (ABE) are represented by straight lines.

Batch fermentation and recovery by gas-stripping

A control batch fermentation experiment (Ferm I) was run with 59.2 g l^{-1} initial glucose concentration in P2 medium using *C. beijerinckii* BA101. Figure 4a shows ABE and acid production profiles by *C. beijerinckii* BA101 over the course of 60 h. The culture produced 5.3 g acetone l^{-1}, 11.9 g butanol l^{-1}, and 0.5 g ethanol l^{-1}, resulting in a total ABE concentration of 17.7 g l^{-1} (Table 1). The maximum cell concentration of 3.2 g l^{-1} was observed at 60 h. At the end of fermentation residual glucose concentration was 14.6 g l^{-1} (Figure 4b). This showed that the culture was unable to utilize all the glucose because of the toxic effect of butanol. The solvent productivity and yield were 0.29 g l^{-1} h^{-1} and 0.40, respectively. At the end of fermentation the acid concentration was 0.9 g l^{-1}.

In order to examine the effect of gas-stripping on the culture, fermentation, and glucose utilization, a batch fermentation experiment (Ferm II) was initiated with initial glucose concentration of 59.7 g l^{-1}. In order to allow cell growth, stationary fermentation was allowed for 15 h, at which time total ABE and cell concentrations were 3 and 2.1 g l^{-1}, respectively. At that stage ABE recovery by gas-stripping was started and the fermentation was allowed to run for 39 h at which time glucose concentration was reduced to 0 g l^{-1} (Figure 5a, b). The maximum cell concentration that was achieved was 4.6 g l^{-1} at this time. It is important to note that at the end of fermentation and recovery, acids were not detected either in the reactor or in condensate, suggest-

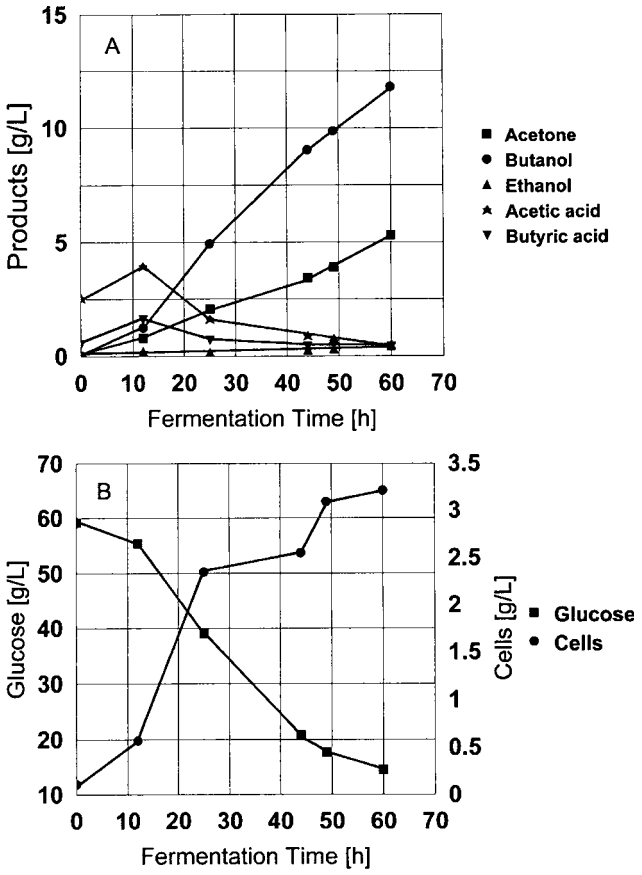

Figure 4. Production of butanol (Ferm I) in a batch reactor using cells of *C. beijerinckii* BA101 containing 60 g l^{-1} glucose solution. (A) Acetone, butanol, ethanol, acetic acid, and butyric acid in fermentation broth at various times; (B) Glucose and cells in fermentation broth at various times.

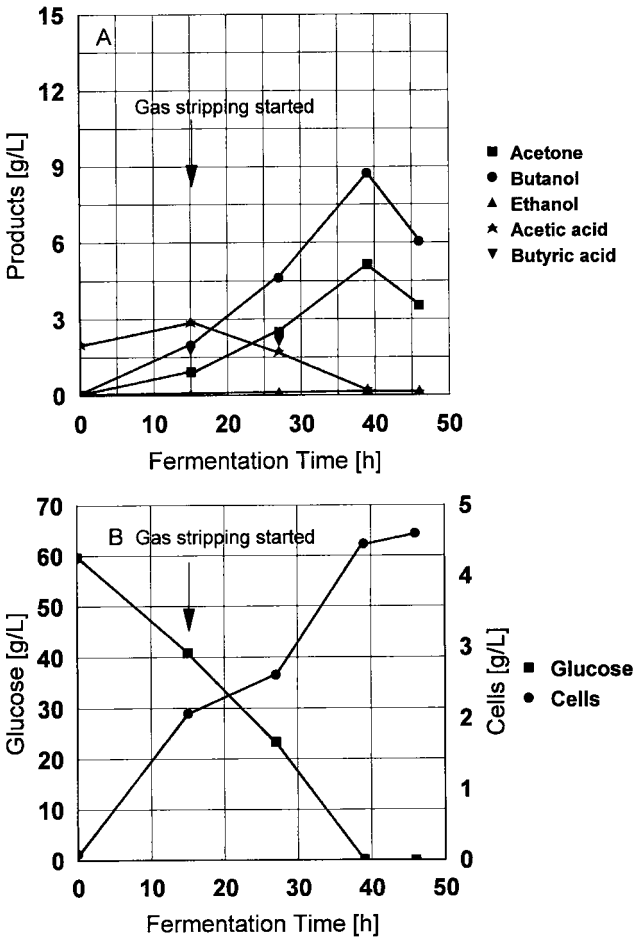

Figure 5. Butanol production (Ferm II) in a batch reactor and *in situ* recovery by gas stripping from 60 g glucose l^{-1} using cells of *C. beijerinckii* BA101. (A) Acetone, butanol, ethanol, acetic acid, and butyric acid in fermentation broth at various times; (B) Glucose and cells at various times in fermentation broth.

Table 1. Production of ABE in non-integrated batch and integrated batch fermentations.

Parameters	Non-integrated batch ferment.[a]	Integrated batch ferment.[a]	Integrated batch ferment.[b]
Acetone (g l^{-1})	5.3	6.9	27.4
Butanol (g l^{-1})	11.9	16.4	46.4
Ethanol (g l^{-1})	0.5	0.3	2.1
Acetic acid (g l^{-1})	0.5	0.0	0.0
Butyric acid (g l^{-1})	0.4	0.0	0.0
Total ABE (g l^{-1})	17.7	23.6	75.9
Total acids (g l^{-1})	0.9	0.0	0.0
Cells (g l^{-1})	3.2	4.6	11.0
Initial glucose (g l^{-1})	59.2	59.7	161.7
Residual glucose (g l^{-1})	14.6	0.0	0.0
Glucose utilized (%)	75.3	100	100
ABE productivity (g l^{-1} h^{-1})	0.29	0.61	0.60
ABE yield (–)	0.40	0.40	0.47

Ferment. – fermentation.
[a] 60 g l^{-1} glucose fermentation.
[b] 161.7 g l^{-1} glucose fermentation.

ing that the system became truly solventogenic. This was also observed by Qureshi & Maddox (1991) when gas stripping was applied to ABE production in an immobilized cell continuous reactor of *C. acetobutylicum* P262. The present experiment demonstrated that during 24 h of gas stripping the mutant strain (*C. beijerinckii* BA101) was not adversely affected.

The total amount of ABE produced was 23.6 g in 39 h (acetone 6.9 g, butanol 16.4 g and ethanol 0.3 g in 1 l reaction volume) resulting in a productivity of 0.61 g l^{-1} h^{-1} and yield of 0.40 (Table 1). It should be noted that this productivity is much higher than achieved in Ferm I. This is because of reduced butanol toxicity and higher cell concentration. During the experiment the glucose utilization rate was 1.53 g l^{-1} h^{-1} (Figure 6). At the end of 46 h (when recovery was stopped) the reaction mixture volume in the reactor was 1000 ml which contained ABE and acetic and butyric acid at 3.5, 6.0, 0.2, 0.0 and 0.0 g l^{-1}, respectively. The total ABE in the reactor was 9.7 g. During 46 h of ABE removal, condensate was recovered three times with volumes 98.6, 79 and 48 ml containing ABE at 42.7, 82.5 and 67 g l^{-1}, respectively. The total amount of condensed ABE was 13.9 g. The ABE selectivities are shown in Figure 3. As compared to the

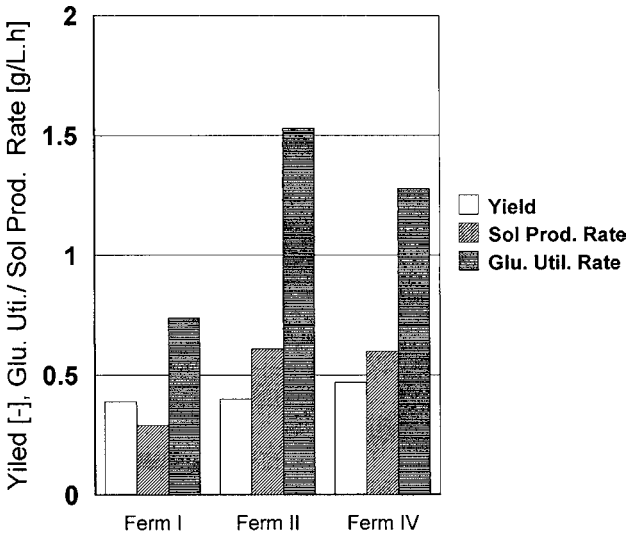

Figure 6. Yield, glucose utilization rate, and ABE (solvent) production rates in various fermentations. For 'Ferm I' see Figure 4; For 'Ferm II' see Figure 5; and For 'Ferm IV' see Figure 7.

selectivities determined with the model butanol solution, reduced selectivities for butanol were observed. A possible reason for these lowered selectivities is the presence of cells in the fermentation broth which was demonstrated in the experiment where 11 g cells l^{-1} were added (Figure 2b) to the model butanol solution. In the present experiment 4.6 g cells l^{-1}were present.

In the next experiment we examined the production of ABE from concentrated sugar solution in order to economize the process of butanol production in the batch process. Concentrated glucose solution (154.1 g l^{-1}) was used to initiate the fermentation (Ferm III). The maximum glucose concentration *C. beijerinckii* BA101 can tolerate and still sustain growth (at a reduced rate due to substrate inhibition) is approximately 165 g l^{-1} (Qureshi & Blaschek 1999a). Use of concentrated sugar solutions reduces process stream volume, thus resulting in improved economics. Owing to initial slow growth due to substrate inhibition, batch fermentation was allowed to proceed uninterrupted for 27 h (data not shown). At that time total ABE and cell concentration were 1 and 0.56 g l^{-1}, respectively. The glucose concentration in the reactor at this time was 141.7 g l^{-1}. After 27 h gas stripping was initiated to recover ABE and continued until 93 h when fermentation stopped leaving behind 59.4 g l^{-1} of unutilized sugar. The total amount of ABE produced was 36.1 g. This represented a productivity and yield of 0.39 g l^{-1} h^{-1} and 0.39, respectively (data not shown).

There are two possible explanations for the cessation of fermentation, one being extended exposure of the cells to gas stripping (and hence agitation) and the other being a lack of nutrients, which will be discussed later. Fermentation data are not provided, because this was not a successful fermentation. However, selectivity data are included in Figure 3 for comparison. During the gas-stripping process, butanol selectivity fluctuated between 13.05 and 6.56 (Figure 3a), acetone selectivity between 5.37 and 9.66 (Figure 3b), and ethanol selectivity between 5.14 and 8.96 (Figure 3c). In this experiment too, acetic and butyric acids were not detected in the condensate. Figure 3 shows that there was no relationship between fermentation broth ABE concentrations and respective selectivities (scattered points).

In order to deduce the cause of cessation of the previous fermentation (Ferm III), attempts were made to initiate another fermentation by pulsing the reactor with nutrients during fermentation and recovery. Hence, we repeated the batch experiment (Ferm IV) using a concentrated sugar solution (161.7 g l^{-1} glucose in P2 medium). Fermentation was carried out undisturbed for an initial 36 h. At that time the cell and ABE concentrations were 0.6 and 1.8 g l^{-1}, respectively (Figure 7a, b). Gas stripping was initiated at that time and continued until 75 h. At this time a reduction in cell concentration was observed. As a result of this observed reduction, a nutrient solution was added to the reactor. 30 ml of stock solutions (10 ml of each of buffer, vitamin and mineral solutions) and 20 ml of yeast extract solution containing 1 g yeast extract were added to the reaction mixture. This amount of nutrients is equivalent to that which is added to 1 l of P2 medium before initiating fermentation. Following nutrient addition fermentation and gas stripping were continued. As seen in Figure 7b a sharp decrease in glucose concen-

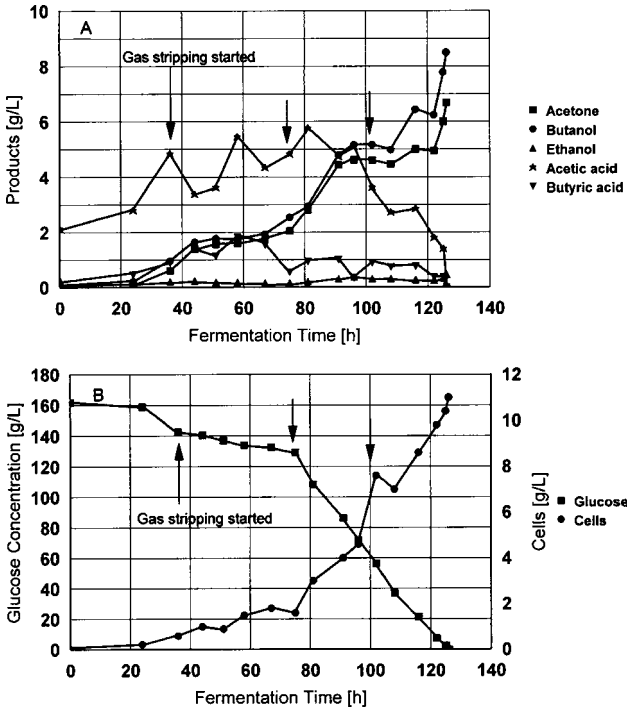

Figure 7. Butanol production (Ferm IV) and *in situ* recovery by gas stripping from 161.7 g l^{-1} glucose solution. (A) Acetone, butanol, ethanol, acetic acid, and butyric acid in fermentation broth at various times; (B) Glucose and cells in fermentation broth at various times. First arrow (from left) shows initiation of gas stripping and the following two arrows show addition of nutrient solution.

tration and an increase in cell concentration were observed suggesting that addition of nutrients was helpful in speeding up the fermentation. A similar dose of nutrients was injected into the reactor at 102 h. As a result of nutrient feeding, the fermentation was completed at 126 h (Figure 7a, b). Due to nutrient feeding the rate of glucose utilization increased from 0.44 g l^{-1} h^{-1} (0–75 h) to 2.53 g l^{-1} h^{-1} (75–126 h). This was due to the fact that cell growth occurred vigorously to a final cell concentration of 11 g l^{-1} when there was no glucose left in the reactor. It should be noted that at 75 h the rate of gas recycle was increased to approximately 9 l min^{-1} as increased cell concentration increases the rate of production and yet hampers the removal of butanol (Figure 2b). This demonstrated that fermentation III failed due to exhaustion of nutrients. Also, this and previous experiments clearly demonstrated that gas stripping did not affect the culture adversely.

At the end of fermentation (126 h) 6.7 g acetone l^{-1}, 8.5 g butanol l^{-1}, 0.5 g ethanol l^{-1}, 0.0 g acetic acid l^{-1}, and 0.0 g butyric acid l^{-1} were present. The reaction mixture volume was 1.26 l. The total amount of ABE present in the reactor was 19.8 g. This amount has been included in the total amount of ABE produced (75.9 g; Table 1). The condensed ABE was 56.1 g. The individual amounts of ABE that the culture produced were 27.4 g acetone, 46.4 g butanol and 2.1 g ethanol. Based on utilization of 161.7 g of glucose this represented a yield of 0.47 which is higher than expected (Table 1). The possible reason for the high yield may be the presence of acetate in the feed medium and the carbon sources available in yeast extract. During fermentation, a total of 6.6 g acetate and 3 g yeast extract was fed to the reactor. Although the culture experienced a long initial lag, a productivity of 0.60 g l^{-1} h^{-1} was achieved in this fermentation. This productivity is comparable to the 60 g l^{-1} glucose fermentation (Ferm II) when integrated with product recovery. Increased cell concentration, the presence of nutrients in the feed medium, and intermittent feeding of nutrients are the reasons for such a high productivity (in spite of substrate inhibition). In this experiment the rate of glucose utilization was 1.28 g l^{-1} h^{-1} (Figure 6). As above (in the integrated experiments) acids were not detected either in the reactor nor in the condensate. The culture utilized all the glucose and acids for growth and solvent production, respectively. Due to the high initial glucose concentration in the reactor, the culture experienced more than 67 h of 'lag phase'.

The selectivities achieved during recovery are shown in Figure 3. During the gas-stripping process, acetone selectivity fluctuated between 4.65 and 10.46, ethanol between 4.74 and 9.32, while that for butanol fluctuated between 6.67 and 13.23. It should be noted that in this recovery experiment acetone (Figure 3b) and butanol (Figure 3a) selectivities followed a similar straight line trend while in case of ethanol there was no trend (Figure 3c). ABE selectivities obtained in integrated fermentations (Ferm III and IV) have been plotted against cell concentrations in the fermentor (Figure 8). It is clearly shown that as ABE concentration increases selectivities of all the three components decrease exponentially.

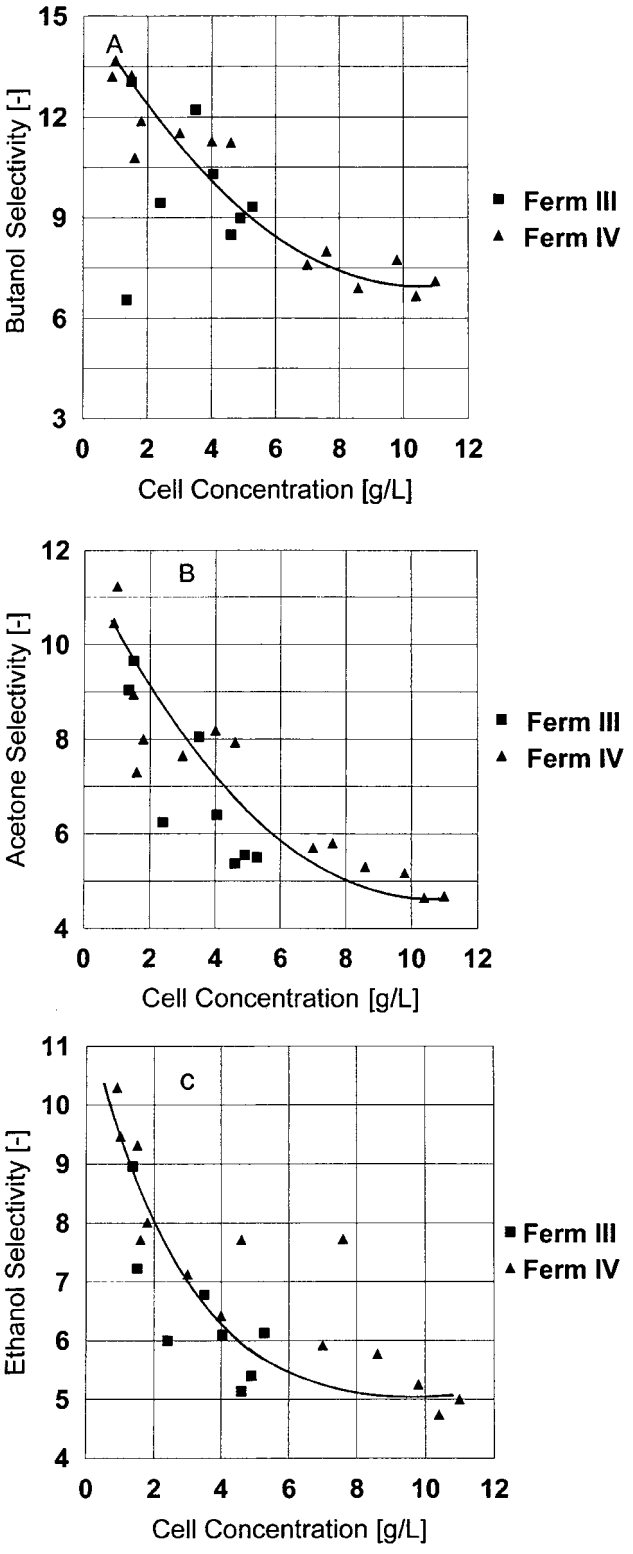

Figure 8. Butanol, acetone, and ethanol selectivities at various cell concentrations in fermentation broth. (A) Butanol; (B) Acetone and (C) Ethanol selectivities. Ferm III (cell concentration not included), Ferm IV (Figure 7).

Discussion

A control batch fermentation experiment with approximately 60 g l^{-1} initial glucose concentration in P2 medium resulted in 14.6 g unused glucose l^{-1}, because of the toxic effect of butanol (Qureshi & Blaschek 2000). To solve this problem of butanol toxicity, the batch fermentation experiment was repeated with the incorporation of *in situ* ABE removal system (gas-stripping). As shown in Figure 5a, b incorporation of gas-stripping resulted in a decrease in fermentation time, and in a complete utilization of glucose and acids in 39 h. Due to decreased product inhibition, cell growth occurred and there was an elevated cell concentration. The increased concentration of cells and decreased inhibition were the reasons for increased productivity (Table 1, Figure 6). The complete absence of acetic and butyric acids in the fermentor at the end of the fermentation time indicated total conversion of these acids into solvents. This shows that solvent removal by gas-stripping promotes acid utilization and conversion to ABE by the culture. More interesting is that gas stripping does not remove acids from the fermentor until they are completely converted to solvent. The average solvent productivity and yield in the integrated system were 0.61 g l^{-1} h^{-1} and 0.39, respectively, compared to the average ABE productivity and yield in the control non-integrated system which were 0.29 g l^{-1} h^{-1} and 0.40, respectively. There was no adverse effect of gas-stripping on the *C. beijerinckii* BA101 mutant culture.

In order to economize the integrated batch fermentation system and reduce the volume of waste disposal streams, we increased the initial glucose concentration to 161.7 g l^{-1} and added nutrients (yeast extract, mineral, buffer and vitamin solutions) to the fermentor when needed (when rate of fermentation or cell growth was low). At the end of the fermentation, the culture utilized all the glucose (161.7 g l^{-1}) in the fermentation broth, produced total ABE of 75.9 g l^{-1} and left no residual acids in the fermentation broth, a condition indicating highly efficient and complete fermentation process (Figure 7a, b). The solvent productivity and yield were increased to 0.60 g l^{-1} h^{-1} and 0.47, respectively. The increase in yield from 0.40 (control) to 0.47 was attributed to utilization of all the acids and carbon present in acetate (in added buffer) and yeast extract. The productivity was comparable to the 60 g l^{-1} glucose integrated fermentation. This was in spite of the fact that the culture experienced more than 67 h of 'lag phase' due to an initial high glucose concentration. The culture growth rate is inhibited above 100 g l^{-1} initial glucose concentration and strongly inhibited above 160–165 g l^{-1} initial glucose level (Qureshi & Blaschek 1999a). Furthermore, the selectivities and ABE concentrations in the condensate were dependent on ABE and cell concentration in the broth. The use of concentrated sugar solution reduces process stream volume, thus economizing the process of biochemical/s production by fermentation. In the present investigation it has been demonstrated that *C. beijerinckii* BA101 can be used in combination with concentrated glucose feed and recovery by gas stripping. In addition to improving the glucose utilization rate in the integrated fermentation, a concentrated product stream is obtained which would require less energy for further recovery. In these studies, condensed ABE concentrations of up to 28.1, 61.8, and 2.0 g l^{-1}, respectively, were achieved depending upon ABE concentrations in the fermentation broth. It should be noted that the selectivities of ABE were reduced due to the presence of cells in the fermentation broth (Figure 8).

Conclusions

ABE were produced in the integrated batch fermentor using gas-stripping as the *in situ* product recovery technique. An integrated batch fermentation experiment with P2 medium containing 60 g glucose l^{-1} produced total ABE of 23.6 g l^{-1} (33% higher) as compared to 17.7 g l^{-1} for the non-integrated process. The fermentation time was also reduced from 60 to 39 h, thereby increasing the productivity. *C. beijerinckii* BA101 produced a total ABE of 75.9 g l^{-1} during the fermentation when using a concentrated sugar solution. Solvent toxicity to the culture and nutrient depletion during long term fermentation processes are two key factors that lead to premature termination of the fermentation. Gas-stripping as a product removal system enhances the selective removal of ABE from the fermentation broth and encourages efficient assimilation of acids produced by the culture for conversion into solvents. During gas-stripping acids were not removed from the fermentation broth. Selectivities of ABE were dependent on ABE concentration and cell concentration in the broth. The culture was not adversely affected by gas stripping. We have demonstrated that *C. beijerinckii* BA101 can use concentrated sugar solutions for butanol production when combined with product recovery by gas stripping.

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References

Annous, B.A. & Blaschek, H.P. 1991 Isolation and characterization of *Clostridium acetobutylicum* mutants with enhanced amylolytic activity. *Applied and Environmental Microbiol*ogy **57**, 2544–2548.

Chen, C.K. & Blaschek, H.P. 1999 Acetate enhances solvent production and prevents degeneration in *Clostridium beijerinckii* BA101. *Applied Microbiology and Biotechnology* **52**, 170–173.

Ennis, B.M., Marshall, C.T., Maddox, I.S. & Paterson, A.H.J. 1986 Continuous product recovery by *in situ* gas stripping/condensation

during solvent production from whey permeate using *Clostridium acetobutylicum*. *Biotechnology Letters* **8**, 725–730.

Ennis, B.M., Qureshi, N. & Maddox, I.S. 1987 Inline toxic product removal during solvent production by continuous fermentation using immobilized *Clostridium acetobutylicum*. *Enzyme and Microbial Technology* **9**, 672–675.

Evans, P.J. & Wang, H.W. 1988 Enhancement of butanol fermentation by *Clostridium acetobutylicum* in the presence of decanol-oleyl alcohol mixed extractants. *Applied and Environmental Microbiology* **54**, 1662–1667.

Formanek, J., Mackie, R. & Blaschek, H.P. 1997 Enhanced butanol production by *Clostridium beijerinckii* BA101 grown in semi-defined P2 medium containing 6 percent maltodextrin or glucose. *Applied and Environmental Microbiology* **63**, 2306–2310.

Garcia, A., Iannotti, E.L. & Fischer, J.L. 1986 Butanol fermentation liquor production and separation by reverse osmosis. *Biotechnology & Bioengineering* **28**, 785–791.

Groot, W.J., van der Lans, R.G.J.M. & Luyben, K.Ch.A.M. 1989 Batch and continuous butanol fermentation with free cells: integration with product recovery by gas stripping. *Applied Microbiology and Biotechnology* **32**, 305–308.

Groot, W.J., van der Lans, R.G.J.M. & Luyben, K.Ch.A.M. 1992 Technologies for butanol recovery integrated with fermentations. *Process Biochemistry* **27**, 61–75.

Groot, W.J., van den Oever, C.E. & Kossen, N.W.F. 1984 Pervaporation for simultaneous product recovery in the butanol/isopropanol batch fermentation. *Biotechnology Letters* **6**, 709–714.

Jesse, T.W., Ezeji, T.C., Qureshi, N. & Blaschek, H.P. 2002 Production of butanol from starch based packing-peanuts and agricultural waste. *Journal of Industrial Microbiology and Biotechnology* **29**, 117–123.

Maddox, I.S. 1988 The acetone–butanol–ethanol fermentation: recent progress in technology. *Biotechnology and Genetic Engineering Reviews* **7**, 189–220.

Maddox, I.S., Qureshi, N. & Roberts-Thomson, K. 1995 Production of acetone–butanol–ethanol from concentrated substrates using *Clostridium acetobutylicum* in an integrated fermentation-product removal process. *Process Biochemistry* **30**, 209–215.

Mollah, A.H. & Stuckey, D.C. 1993 Feasibility of *in-situ* gas stripping for continuous acetone-butanol fermentation by *Clostridium acetobutylicum*. *Enzyme and Microbial Technology* **15**, 200–207.

Nielson, L., Larsson, M., Holst, O. & Mattiasson, B. 1988 Adsorbents for extractive bioconversion applied to the acetone butanol fermentation. *Applied Microbiology and Biotechnology* **28**, 335–339.

Qureshi, N. & Blaschek, H.P. 1999a Production of acetone butanol ethanol (ABE) by a hyper-producing mutant strain of *Clostridium beijerinckii* BA101 and recovery by pervaporation. *Biotechnology Progress* **15**, 594–602.

Qureshi, N. & Blaschek, H.P. 1999b Butanol recovery from model solutions/fermentation broth by pervaporation: evaluation of membrane performance. *Biomass & Bioenergy* **17**, 175–184.

Qureshi, N. & Blaschek, H.P. 2000 Butanol production using *Clostridium beijerinckii* BA101 hyper-butanol producing mutant strain and recovery by pervaporation. *Applied Biochemistry and Biotechnology* **84**, 225–235.

Qureshi, N. & Blaschek, H.P. 2001 Recovery of butanol from fermentation broth by gas stripping. *Renewable Energy* **22**, 557–564.

Qureshi, N. & Maddox, I.S. 2003 Production of butanol from concentrated lactose/whey permeate using *Clostridium acetobutylicum* and removal by perstratction. 25th Biotechnology Conference for Fuels and Chemicals, Breckenridge, CO, USA. May 4–7.

Qureshi, N. & Maddox, I.S. 1991 Integration of continuous production and recovery of solvents from whey permeate: use of immobilized cells of *Clostridium acetobutylicum* in fluidized bed bioreactor coupled with gas stripping. *Bioprocess Engineering* **6**, 63–69.

Qureshi, N., Maddox, I.S. & Friedl, A. 1992 Application of continuous substrate feeding to the ABE fermentation: relief of product inhibition using extraction, perstraction, stripping, and pervaporation. *Biotechnology Progress* **8**, 382–390.

Qureshi, N., Meagher, M.M. & Hutkins, R.W. 1999 Recovery of butanol from model solutions and fermentation broth using a silicalite/silicone membrane. *Journal of Membrane Science* **158**, 115–125.

Qureshi, N., Meagher, M.M., Huang, J. & Hutkins, R.W. 2001 Acetone butanol ethanol (ABE) recovery by pervaporation using silicalite–silicone composite membrane from fed-batch reactor of *Clostridium acetobutylicum*. *Journal of Membrane Science* **187**, 93–102.